(35.9%) and 8 (20.51%) pts had a HLA mismatched donor. Therapy associated complications identified included hyperacute GVHD (13pts [33.3%]), acute renal failure (13 pts [33.3%]), hemolysis (14 pts [35.9%]), neuro-toxicity (7 pts [17.95%]), cytopenias (10 pts [25.6%]), dyslipidemia (6 pts [15.4%]) and skin changes (2 pts [5.13%]). 15 pts (38.5%) had GrIII-IV GVHD. 12 pts (30.8%) died of who 3 (25%) were directly related to the siro-tac associated toxicity.

Conclusions: We conclude that the sirolimus-tacrolimus combination GVHD prophylaxis regimen, is associated with considerable toxicities that contribute to the transplant associated morbidity and mortality in the allogeneic hematopoietic stem cell transplantation setting, not attributable to the conditioning regimen. Therefore this regimen should be used cautiously outside of a clinical trial.

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RESTING REGULATORY T LYMPHOCYTES: A BIOMARKER FOR CHRONIC GVHD

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T cell depletion and peri-transplant serotherapy have reduced the incidence and severity of acute graft versus host disease (GVHD); however, chronic GVHD continues to be a major clinical limitation to the increased use of hematopoietic stem cell transplantation (HSCT). Deficiencies in naturally occurring regulatory T lymphocytes (Treg) have been hypothesized to contribute to chronic GVHD. The evaluation of the role of Treg lymphocytes in the pathogenesis of chronic GVHD have been limited by a lack of well-defined immunophenotypic characterization of Treg lymphocytes. Most previous evaluations of Treg lymphocytes in HSCT recipients, which used either CD25 and/or FoxP3 expression, were confounded by the fact that activated conventional T lymphocytes (Tcon) expressed CD25 and FoxP3. We have performed a cross-sectional analysis of pediatric HSCT recipients using the Miyara classification of Treg lymphocytes. (Immunity 30:899, 2009) The Miyara classification can distinguishes between Treg lymphocytes, both resting and activated, and activated Tcon lymphocytes, permitting a more accurate assessment of Treg in the HSCT setting. Pediatric HSCT recipients more than 1 year post-HSCT were evaluated for their resting (r) and activated (a) Treg lymphocytes and their r/a ratio determined. (Table 1)

Table 1. Resting and Activated Treg Lymphocytes in HSCT Recipients

	rTreg	aTreg	
Patient	(% of total CD4 T lymphocytes x 10^{-1})		r/a ratio
Allogeneic-			
No hx of acute/chronic GVHD	17.3	6.2	4.7
Active chronic GVHD	6.4	1.8	3.5
Hx of chronic GVHD	14.0	6.8	3.2
Autologous	11.3	5.2	2.2
Normal Individuals	19.3	3.6	3.9

Allogeneic HSCT recipients with no history of either acute or chronic GVHD did not differ from normal individuals. Neither did allogeneic recipients with only a history of acute GVHD nor the recipients of autologous HSCT. Allogeneic recipients with active chronic GVHD had reductions in both the percentage of resting Treg lymphocytes ($p = .02$ compared to normal or recipients with neither acute nor chronic GVHD) and of activated Treg lymphocytes ($p = .002$ compared to recipients without acute or chronic GVHD and $p = .0008$ compared to normals). Allogeneic recipients, who have had the clinical resolution of their chronic GVHD, did not differ from normal individuals. The clinical resolution of chronic GVHD is associated with a normalization of both resting and activated Treg lymphocytes. The longitudinal evaluation of resting Treg levels may aid in the management of chronic GVHD patients and may predict when clinical improvement occurs.

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BORTEZOMIB FOR STEROID REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE (GVHD)

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Steroid refractory GVHD remains a significant problem after allogeneic HSCT with a mortality rate exceeding 90% in some series and no standard for salvage treatment. Murine data suggest bortezomib inhibits acute GVHD, but preserves graft-versus-tumor effect. We sought to test whether the same activity could be demonstrated in a clinical setting. Patients were eligible for the trial if they had clinical or histological evidence of acute GVHD and had been treated with 2mg/kg/day of methylprednisolone and had 1) either a minimum of 3 days of corticosteroids with progressive disease or 2) a minimum of 7 days without a response or 3) had progression when steroids were tapered to less than 2mg/kg/day of methylprednisolone. Bortezomib was given at a dose of 1.3mg/m2 administered as a bolus intravenous injection twice weekly for 2 weeks (days 1, 4, 8, 11) followed by a 10 day rest period constituting one 21 day treatment cycle. A complete response (CR) was defined as no clinical or laboratory signs of acute GVHD in all organs, and a partial response (PR) required at least a 1 grade reduction in overall GVHD scoring in at least 1 organ system without worsening in others. Response was determined on day 21 after the first cycle and every 3 weeks until 3 months after the last cycle or death whichever came first. After the 1st cycle, patients achieving CR or PR were treated with additional cycles until they had a dose limiting toxicity or a complete response, and patients without response were taken off study and considered treatment failure. Three of 11 patients treated were not evaluable for efficacy; 1 withdrew from the study after less than 1 cycle without signs of toxicity, 1 died 1 week after receiving the 1st cycle of septic shock, and the 3rd patient developed a concomitant viral infection that could not be distinguished from possible concomitant GVHD. Of the 8 evaluable patients 4 had no response and 4 had at least a partial response. In the 4 patients with responses 2 were complete responses and 2 were partial. Ten of the 11 patients have died, 2 of progressive disease, 6 of infection, 1 of hemorrhage and 1 of pulmonary toxicity. Whether bortezomib contributed to any of these deaths was unclear. One of the partial responders was taken off the study for peripheral neuropathy and is still alive 4.5 years later. In this small study bortezomib demonstrated some activity in steroid refractory acute GVHD with some neurotoxicity.

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LATENT CMV AND THE CD4+ T CELL TRANSCRIPTION PROGRAM IN PATIENTS WITH CHRONIC GVHD

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Latent CMV infection is known to have a life long effect on the distribution of T cell subsets, but little is known about the impact on cell function. We performed a gene expression analysis in purified CD4+ T cells from 68 hematopoietic cell transplant (HCT) recipients (median age 48; range 20-65) studied on average 5 years after HCT (median 4.75; range 1-20 years). The study population included 38 patients with active chronic GVHD (cGVHD) and 30 tolerant (TOL) patients. Tolerance was defined by absence of signs, symptoms of cGVHD and immunosuppressive therapy (IST) for 3 months. Gene expression was measured on Illumina bead arrays. CMV status was defined by pre-transplant recipient CMV serology (by ELISA). There was no recorded evidence of CMV reactivation at the time of study. Nine of 93 candidate genes associated with immune function and inflammation were found to be associated CMV serostatus in cGVHD patients at a significance threshold of $p<0.05$, but only four genes (ITK; CD86; PLCG1; PIK3CB) were

associated with CMV serostatus in TOL patients. A multivariate analysis including: acute GVHD, conditioning regimen, marrow vs. PBSC; related vs. unrelated donor, matched vs. mismatched donor, recipient age and time post-HCT was performed Table 1.

cGVHD patients had a profile consistent with T effector cell activation that was not present in TOL.

The characteristic inflammatory environment, increased cytokine production, immunosuppressive therapy and impaired T cell immune reconstitution observed in cGVHD, may increase the risk of CMV reactivation and subsequent upregulation of genes related to T cell activation and effector functions. In contrast, tolerant patients may have achieved greater immune reconstitution and more effective CMV surveillance and control.

T cell activation during cGVHD is a complex process that appears to be influenced by latent CMV.

Table 1. Multivariate analysis

	Active cGVHD	Tolerant
	CMV+ (n = 17) vs CMV-(n = 21)	CMV+ (n = 11) vs CMV-(n = 19)
ITK		0.023↑
CD86		0.034↑
PLCG1		0.033↑
PIK3CB		0.005↑
GZMB	0.024↑	
INFg	0.015↑	
IL4R	0.025↓	
PTPN7	0.000↑	
PRF1	0.000↑	
GZMA	0.003↑	
PDCD1	0.008↑	
IL12RB1a	0.016↑	
IL12RB1b	0.000↑	

↓downregulated gene
↑upregulated gene

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INDUCTION OF A GVHD-LIKE SYNDROME FOLLOWING ALLOGENEIC BMT AND CsA THERAPY

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Cyclosporine A (CsA) is a calcineurin inhibitor that is used to prevent graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell (HSCT)/bone marrow transplantation (BMT). While CsA is utilized for the prophylaxis of GVHD, many patients develop GVHD while on, or after removal from therapy. A disorder develops with clinical symptoms and pathology that is similar to allogeneic GVHD after syngeneic BMT and CsA therapy and has been termed, syngeneic GVHD (SGVHD). In mice, SGVHD is mediated by $CD4^+$ T cells and required an intact host microbiota for the disease to develop. Interestingly, it was recently shown that antibiotic-responsive intestinal inflammation developed after cord blood stem transplantation and treatment with calcineurin inhibitors as prophylaxis for the development of GVHD. Data will be presented that demonstrates the induction of a GVHD-like disease following allogeneic BMT and CsA therapy in mice. We have termed this disease CsA-induced pseudoGVHD (PGVHD). In this inducible disease, pathology characteristic of GVHD occurs in the colon and liver. Similar to the SGVHD model, increased $CD4^+$ T cell responsiveness against microbial antigens is associated with an increase in T_H1/T_H17 immunity relative to control BMT animals. The mechanism(s) responsible for the induction of PGVHD are unknown. Whether the development of this disorder is due to incomplete immunosuppression, altered immune regulation, generation of immunity against microbial antigens or a combination of these possibilities is currently under investigation. The PGVHD model will shed light on this question with an eye towards future development of novel therapies to address this issue clinically.

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SYSTEMIC EFFECTS OF ORAL BUDESONIDE IN HEMATOPOIETIC TRANSPLANT: IMPLICATIONS OF DRUG INTERACTION WITH AZOLES

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Oral budenoside (BUD) is commonly used as a topical adjunct in graft-versus-host-disease (GVHD) of the gastrointestinal tract. BUD possesses a high ratio of topical-to-systemic activity that limits steroid toxicity. It is, however, inactivated via cytochrome P450 isoenzymes so that concomitant administration of azoles which compete for this enzyme increases its bioavailability. The clinical significance of such increased bioavailability after hematopoietic stem cell transplantation is unknown.

The development of iatrogenic Cushing's syndrome in a patient receiving allogeneic hemopoietic stem cell transplantation (alloHSCT) who was taking BUD as the sole source of steroids, led us to explore the frequency of Cushing's disease or other manifestations of significant systemic exposure to exogenous steroids in patients who had received alloHSCT and were receiving both BUD and an azole drug. We reviewed the medical charts of the 60 patients who received an alloHSCT between January 2008 and August 2011. Five patients (Table-1) met the criteria of diagnosis of acute or chronic GVHD; continuation of BUD for a further 21 days or more after cessation of systemic steroids; and concomitant administration of an azole drug. The charts were reviewed for evidence of clinical development of iatrogenic Cushing's syndrome and for evidence of abnormalities in morning plasma cortisol levels and serum glucose levels.

BUD was initiated at 9 mg per day and the dose was titrated according to response. All these patients were receiving either fluconazole (n = 3) or voriconazole (n = 2) for fungal prophylaxis.All had suppression of morning plasma cortisol consistent with systemic absorbtion and significant plasma levels of the topically applied drug. The patients also had rapid (within 3 weeks) onset of iatrogenic Cushing's syndrome, with median weight gain of 5.7 kg (range = 4-7.1 kg). All patients developed facial mooning, central obesity and glucose intolerance. Plasma cortisol remained at 1 ug/dl even when BUD was titrated to 3mg/day, at which time exacerbation of GUT and/or skin GvHD was seen, necessitating dose escalation. Absolute neutrophil and lymphocyte counts remained unchanged with no increased infections.

Hence the effect of combining azole-drugs with BUD is to consistently increase systemic levels of the steroid sufficiently to induce Cushing's syndrome, reducing the selective benefit of this "topical" agent for gut GvHD in patients receiving both agents.

Table 1. Patients characteristics on BUD alone

Patient/transplant	GVHD/ Treatment	BUD alone duration (days)	Cushing's manifestation
52, M, AML, MIUD in CR-2, BU/CY/AL, tacrolimus	Grade II aGvHD, skin, gut, liver, Limited cGvHD, skin, gut, Prednisone, BUD	571*	Plasma cortisol = 1 ug/dl, weight gain = 5.7 kg, facial mooning, central obesity, glucose intolerance
51, M, AML, MRD in active disease, BU/CY, MTX, tacrolimus	Grade II aGvHD, skin,gut, prednisone, BUD	62	Plasma cortisol = 1ug/dl, weight gain = 4 kg facial mooning, central obesity, glucose intolerance
67, M, AML, MUD in CR-2, FL/MEL/AL, tacrolimus	Grade II aGvHD, skin, gut, Limited cGvHD, skin, gut, prednisone, BUD	151*	Plasma Cortisol = 1ug/dl, weight gain = 4.7 kg, facial mooning, glucose intolerance

(*Continued*)